

Comparative Spermatology of Anurans with Special References to Phylogeny

Ae Sook KWON & Young Hwan LEE

Department of Biology Education, Taegu University, Kyungsan 713-714, Korea

ABSTRACT

The anurans so far examined can be divided into three groups based on the data of comparative spermatology. The first group, Ascaphidae and Discoglossidae, is considered to be plesiomorphic because it has most plesiomorphic characters, endonuclear canal, rod-shaped endonuclear perforatorium and mitochondria adjacent to only the axial rod as in urodeles. The second group, including Myobatrachidae, Bufonidae, Hylidae and Leptodactylidae is characterized by a conical acrosome and a conical extranuclear perforatorium. However, Myobatrachidae differ from the other three families, in mitochondrial position and centriolar arrangement. These characteristics are rather similar to those of ascaphids, discoglossids and urodeles. Thus Myobatrachidae appears to be the most primitive among this group. The tail of the first two groups consists of an axoneme, axial rod and undulating membrane. However it contains an axoneme and axial rod in *Hyla*, and an axoneme only in *Telmatobius*. The third group including Pipidae, Ranidae and Rhacophoridae is characterized by a 140° angle of centrioles and a tail with only the axoneme. These features might represent a synapomorphy for this group. The main evolutionary pathways observed in anurans are: 1) a reduction in the length of the endonuclear canal and the eventual disappearance of this structure; 2) the disappearance of the subacrosomal cone and the perforatorium; 3) the disappearance of the undulating membrane and the axial rod; and 4) an increase in the angle between the two centrioles. Phylogenetic conclusions drawn from sperm ultrastructure coincide in many points with previous data based on somatic features.

RÉSUMÉ

Spermatologie comparée des Anoures et rapports avec la phylogénie

Les Anoures examinés jusqu'ici peuvent être divisés en trois groupes en fonction de la spermatologie comparée. Le premier groupe, les Ascaphidae et Discoglossidae, est considéré comme plésiomorphe parce qu'il possède la plupart des caractères plésiomorphes: canal endonucléaire, perforatorium endonucléaire en forme de baguette et mitochondrie adjacente seulement à la baguette axiale, comme chez les Urodèles. Le second groupe, comprenant les Myobatrachidae, Bufonidae, Hylidae et Leptodactylidae est caractérisé par un acrosome conique et un perforatorium extranucléaire conique. Toutefois, les Myobatrachidae diffèrent des trois autres familles par la position de la mitochondrie et la disposition des centrioles. Ces caractéristiques sont assez proches de celles des Ascaphidae, Discoglossidae et Urodèles. Les Myobatrachidae semblent donc être les membres les plus primitifs de ce groupe. La queue, dans les deux premiers groupes, consiste en un axonème, une baguette axiale et une membrane ondulante. Toutefois, elle contient seulement un axonème et une baguette axiale chez *Hyla*, et seulement un axonème chez *Telmatobius*. Le troisième groupe, comprenant les Pipidae, Ranidae et Rhacophoridae, est caractérisé par un angle de 140° des centrioles et une queue contenant seulement l'axonème. Ces caractéristiques pourraient représenter une synapomorphie pour ce groupe. Les étapes évolutives principales observées chez les Anoures sont 1) une réduction de la longueur du canal endonucléaire et la disparition finale de cette structure; 2) la disparition du cône subacrosomien et du perforatorium; 3) la disparition de la membrane ondulante; et 4) l'augmentation de l'angle entre les deux centrioles. Les conclusions phylogénétiques tracées à partir de l'ultrastructure des spermatozoïdes coïncident en de nombreux points avec les données précédentes basées sur les caractéristiques somatiques.

Amphibia are divided into the three orders Apoda, Urodela and Anura. The Anura is often considered as a super-order [30]. Anura can be subdivided into two distinct levels of organization, with primitive and higher families, on morphological data. However, the phyletic relationships between and within the two levels are extremely difficult to clarify because anurans are morphologically quite conservative.

The ultrastructure of the spermatozoon has been the subject of considerable study in the past twenty years in several animal groups and is recognized as an important indicator of phylogenetic relationships [6, 15, 21, 28, 31]. Anuran spermatozoa provide a useful suite of taxonomic characters because they have sufficient variety in the structure of the acrosome, perforatorium, and tail, and the arrangement of the centrioles. Despite the taxonomic value of sperm structure, there have been little data available on phylogeny of anuran spermatozoa.

The ultrastructure of the spermatozoon is now known in 31 species (17 genera) of anurans [12-13, 18, 22-27, 29, 34-39]. This enables us to give a summary of the ultrastructure of the spermatozoon and its phylogeny.

The purpose of this chapter is to describe the ultrastructure of spermatozoa in six species of four families of anurans and to compare it to those of other groups of amphibians. The results are also discussed with regard to the phylogenetic position.

MATERIALS AND METHODS

Spermatozoa from the following anuran species were used in this study (asterisks indicate new data); *Ascaphus truei* (Ascaphidae); *Discoglossus pictus*, *Alytes obstetricians*, *Bombina variegata*, **B. orientalis* (Discoglossidae); *Adelotus brevis*, *Limnodystes peronii*, *Mixophyes fasciolatus*, *Neobatrachus pelobatoides* (Myobatrachidae); *Bufo arenarum*, *B. marinus*, **B. bufo gargarizans* (Bufonidae); *Odontophrynus cultripes*, *Telmatobius hauthali* (Leptodactylidae); *Litoria caerulea*, *L. fallax*, *L. gracilentia*, *L. lesueuri*, *L. peronii*, *L. rubella*, *Pachymedusa dacnicolor*, **Hyla japonica*, *H. meridionalis* (Hylidae); *Xenopus laevis* (Pipidae); *Rana clamitans*, *R. pipiens*, **R. nigromaculata*, **R. dybowskii*, **R. rugosa* (Ranidae); *Rhacophorus arboreus*, *R. schlegelii* (Rhacophoridae).

Species of anurans were collected in the neighbourhood of Taegu, Korea. Testes were dissected and fixed in 2.5%-5% glutaraldehyde in 0.1M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide in the same buffer. They were then dehydrated in a graded ethanol series and embedded in Epon 812. The samples were sectioned on a Sorvall MT 2-B ultramicrotome, stained in 4% aqueous uranyl acetate, post-stained with lead citrate and examined with a Hitachi H-600 electron microscope.

OBSERVATIONS

Bombina orientalis

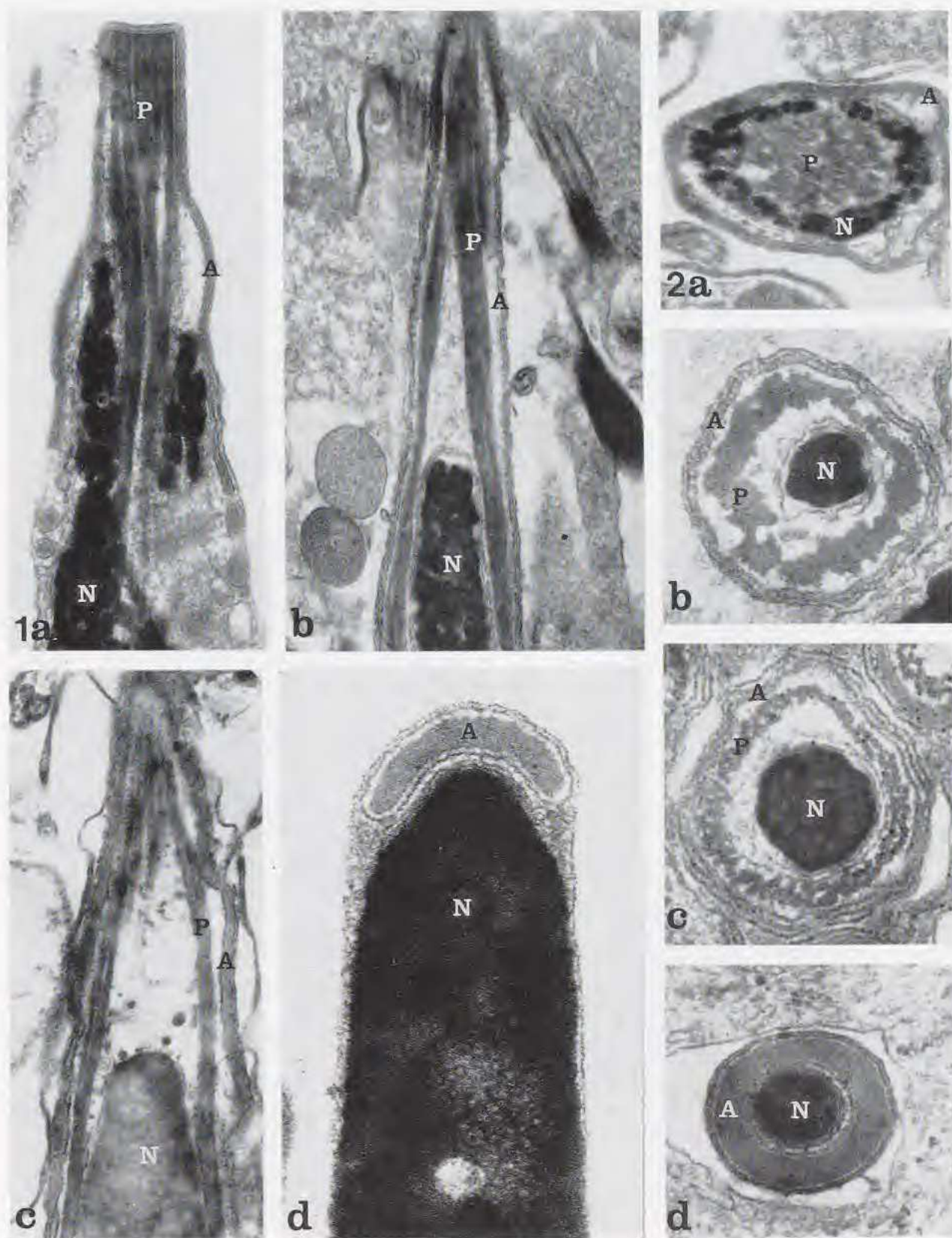
B. orientalis possesses a spermatozoon with a peculiar architecture unlike the classical sequence of acrosome, nucleus and flagellum. The flagellum is juxtaposed longitudinally along the nucleus. The nucleus is cone shaped and some nuclear lacunae, irregular in shape, are scattered within the nucleus. The chromatin is not completely compact but condensed into large masses (Fig. 1a).

The acrosome consists of a thin vesicle truncated at the anterior end and its material is homogenous and moderately electron dense. The subacrosomal space contains the perforatorium

FIG. 1. — Longitudinal sections of head. **a:** *Bombina orientalis*, x 30 000; **b:** *Bufo bufo gargarizans*, x 35 700; **c:** *Hyla japonica*, x 44 000; **d:** *Rana dybowskii*, x 75 000.

FIG. 2. — Transverse sections of head. **a:** *Bombina orientalis*, x 30 000; **b:** *Bufo bufo gargarizans*, x 56 000; **c:** *Hyla japonica*, x 63 000; **d:** *Rana rugosa*, x 52 000.

Abbreviations used in the figures: A, acrosome; AR, axial rod; Ax, axoneme; C, centriole; EC, endonuclear canal; FS, fibrogranular sheet; M, mitochondria; MF, marginal filament; N, nucleus; P, perforatorium; PM, pericentriolar material; UM, undulating membrane; V, vesicle.



which emerges from an endonuclear canal in the tip of the nucleus (Figs 2a, 3a). The perforatorium consists of bundles of filamentous material arranged parallel to each other.

The neck region is located in the lateral surface of the nucleus which is furrowed by a longitudinal depression which contains an anterior portion of the axial rod and proximal centriole (Fig. 3a, b). Two centrioles form an angle of 70° , approximately, to each other and the proximal centriole is perpendicular to the longitudinal axis.

The tail contains an axoneme, axial rod, undulating membrane and mitochondria. The axoneme, of the typical 9+2 pattern, is lateral to the main axis which is occupied by the axial rod. The axial rod extends almost to the tip of the flagellum. The undulating membrane is short in the proximal region of the tail and becomes longer toward the middle region. Mitochondria are arranged in a semicircle and positioned only around the axial rod (Fig. 4a).

Bufo bufo gargarizans

The nucleus is cylindrical in shape and the chromatin is completely condensed. The base of the nucleus holds an implantation fossa in which the proximal centriole lodges.

The acrosome consists of a very thin vesicle containing homogenous material of moderate electron density as in *B. orientalis* (Fig. 1b). The subacrosomal space contains the perforatorium consisting of bundles of microfilaments. The perforatorium runs along the inner acrosomal membrane, unlike *B. orientalis* (Fig. 2b). An endonuclear canal is not present in this species.

In the neck region two centrioles are perpendicular to each other and embedded in pericentriolar material (Fig. 3c). The pericentriolar material connecting with the anterior end of the axial rod shows transverse striations.

The tail contains an axoneme, axial rod, undulating membrane and mitochondria. The axial rod which is associated with axonemal doublet no. 3 extends to the principal piece of the flagellum (Fig. 5a). The end piece contains only the axoneme. Mitochondria constituting a sheath are separated from the flagellum by the cytoplasmic canal and surround the axoneme, axial rod and undulating membrane (Fig. 4b).

Hyla japonica

The spermatozoon of *H. japonica* has an ultrastructure very similar to that of *B. bufo gargarizans* (Figs 1c, 3d). Differences between two will be noted here.

The spermatozoa are shorter and more slender than those of *B. bufo gargarizans*. The perforatorium consists of microtubule-like arrays instead of the microfilaments in other anuran spermatozoa (Fig. 2c). No transverse striations appear in the pericentriolar material.

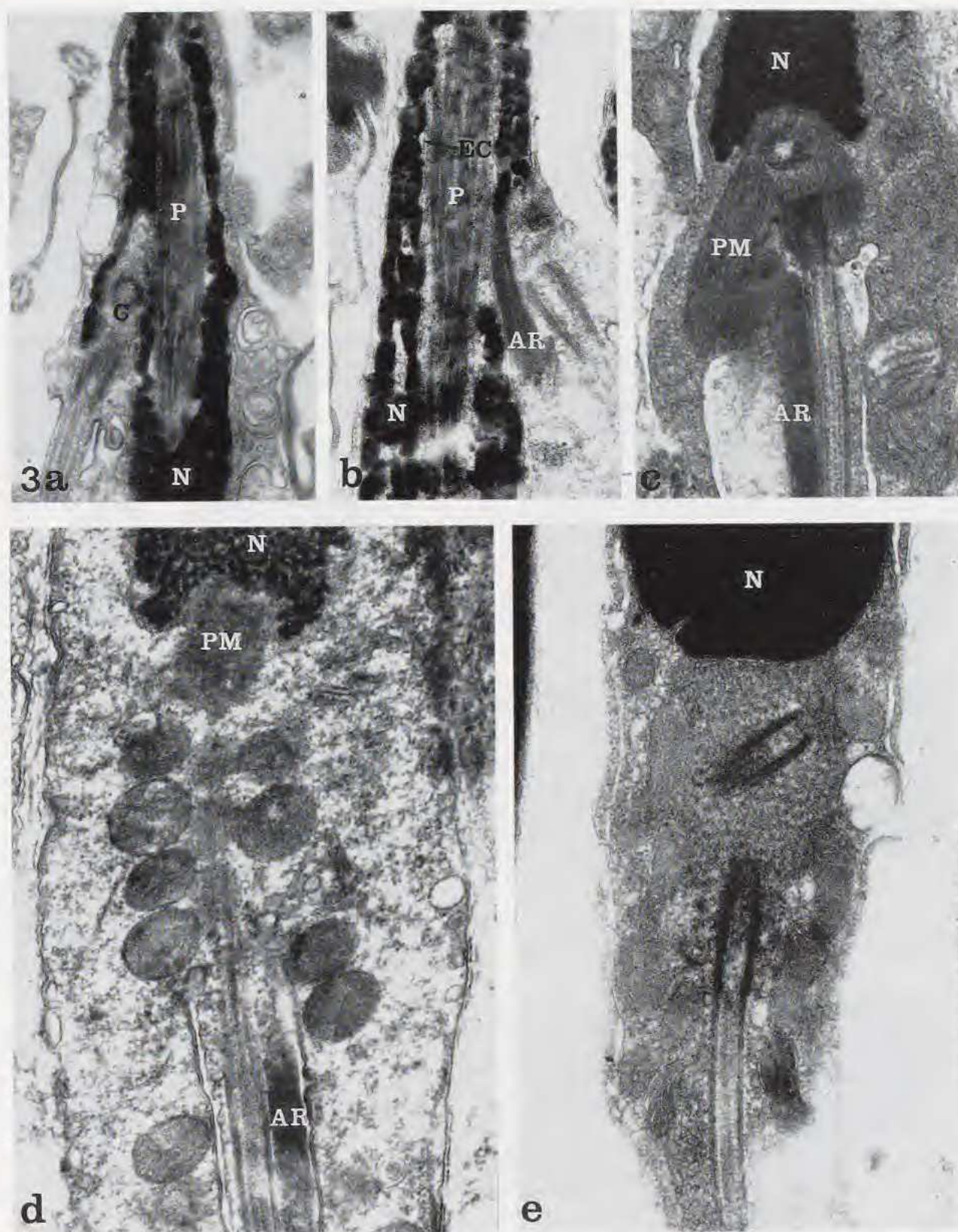
The tail contains an axoneme and axial rod without undulating membrane. The axial rod is closer to the axonemal doublet no. 3 in the middle and principal pieces (Figs 4c, 5b) but disappears in the distal region of the flagellum.

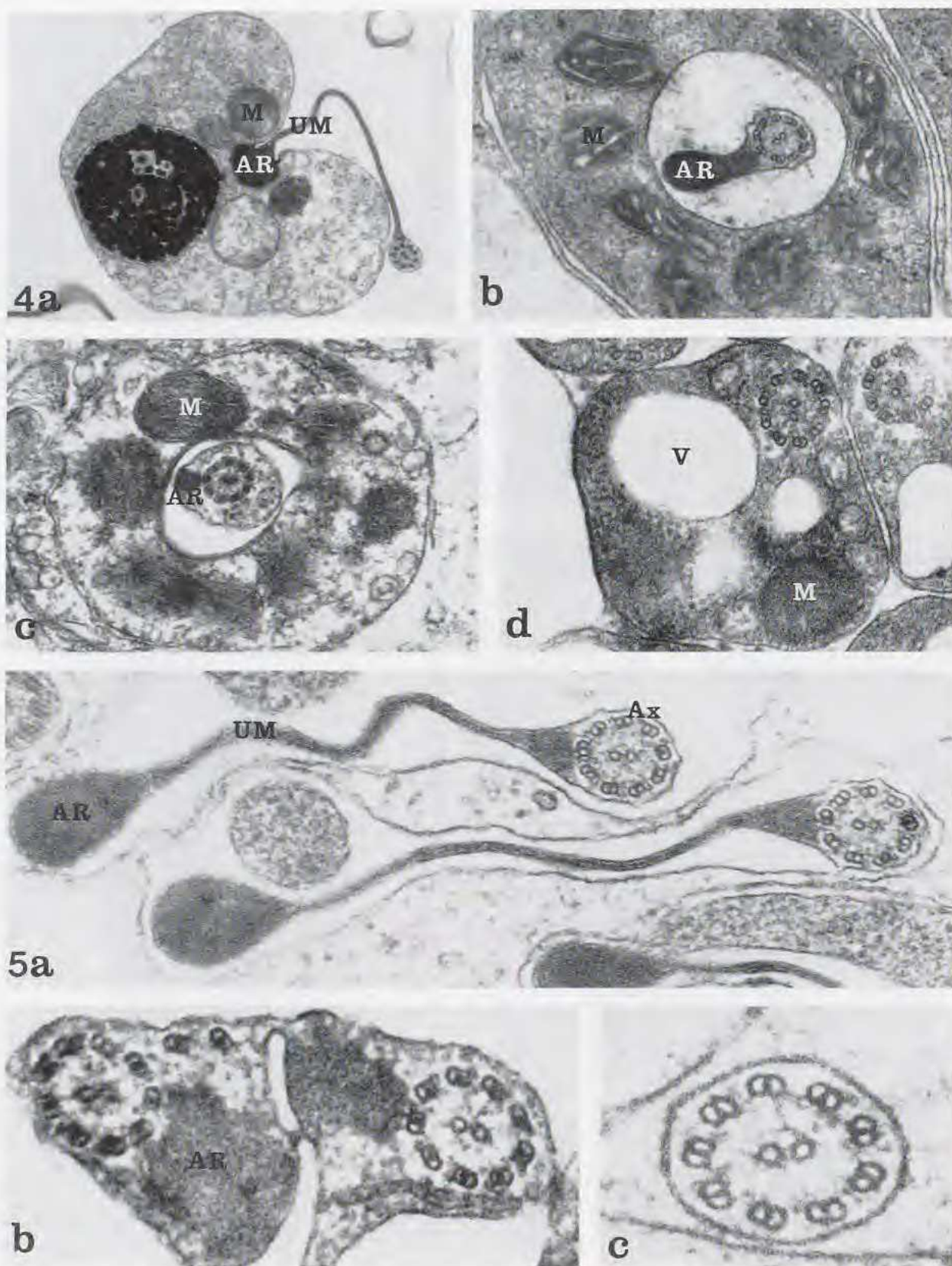
Rana nigromaculata, *Rana dybowskii*, *Rana rugosa*

The spermatozoa of all three species examined have a very similar ultrastructure except the pericentriolar material.

The nucleus, with compact chromatin, is cylindrical and slightly tapered at both ends. The posterior end of the nucleus has an implantation fossa in which the centrioles do not reside. Intranuclear inclusions are visible within the nucleus.

FIG. 3. — Longitudinal sections of head-tail junction. **a**, *Bombina orientalis*, x 23 000; **b**, *Bombina orientalis*, x 30 000; **c**, *Bufo bufo gargarizans*, x 34 000; **d**, *Hyla japonica*, x 30 000; **e**, *Rana rugosa*, x 36 000.





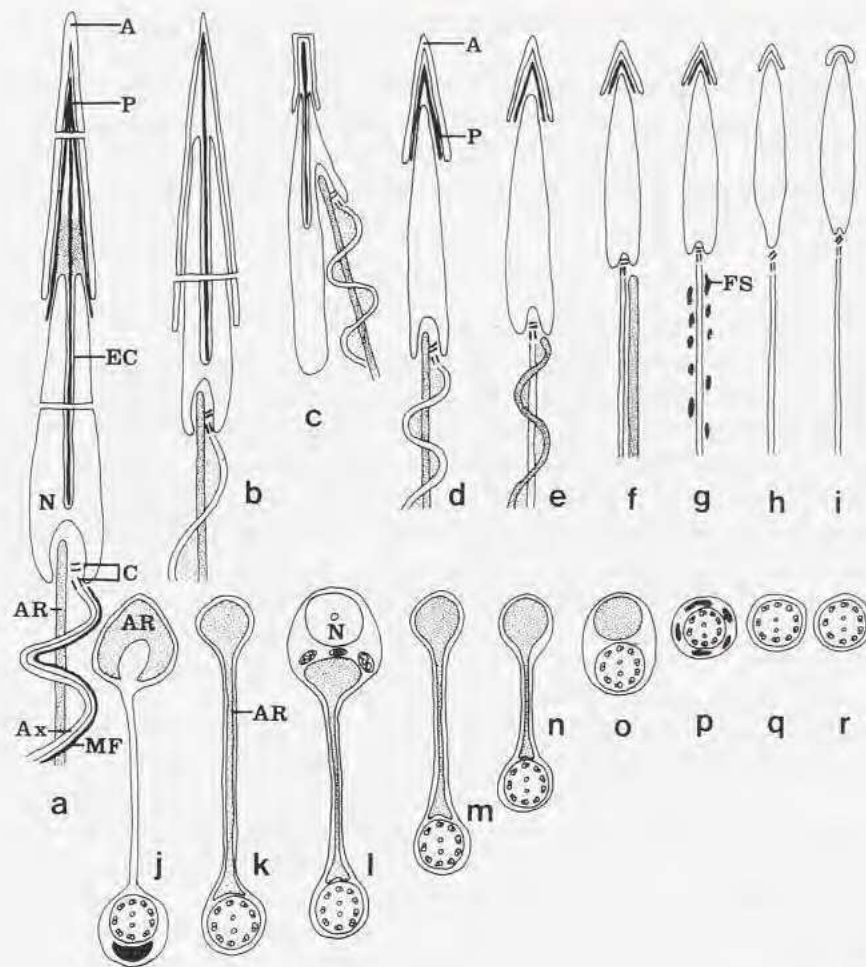


FIG. 6. — Diagrammatic representation of sections of amphibian spermatozoa. **a-i**: longitudinal sections; **j-r**: cross sections of the principal piece. **a, j**: Salamandridae, *Pleurodeles* [32]. **b, k**: Discoglossidae, *Discoglossus* [39]. **c, l**: Discoglossidae, *Bombina* [23]. **d, m**: Myobatrachidae, *Limnodynastes* [25]. **e, n**: Leptodactylidae, *Odontophrynus* [12]. Bufonidae, *Bufo* [24]. Hylidae, *Pachymedusa* [37], Hylidae, *Litoria* [26]. **f, o**: Hylidae, *Hyla* [27]. **g, p**: Leptodactylidae, *Telmatobius* [34]. **h, q**: Pipidae, *Xenopus* [13]. **i, r**: Ranidae, *Rana*.

The acrosome is a saclike structure situated at the most anterior portion of the nucleus (Fig. 1d). It is shorter and thicker than in other species mentioned above. There is no perforatorium nor an endonuclear canal (Figs 1d, 2d).

The neck region contains the centrioles, pericentriolar material and mitochondria (Fig. 3e). Two centrioles form an angle of approximately 140° and the distal centriole is parallel to the main axis. Two centrioles are situated outside the nuclear fossa. Mitochondria aggregate around the base of nucleus, the centrioles and the axoneme. A cytoplasmic canal is not observable.

FIG. 4. — Transverse sections of middle piece. **a**: *Bombina orientalis*, x 21 000; **b**: *Bufo bufo gargarizans*, x 42 000; **c**: *Hyla japonica*, x 40 000; **d**: *Rana dybowskii*, x 70 000.

FIG. 5. — Transverse sections of principal piece. **a**: *Bufo bufo gargarizans*, x 72 000; **b**: *Hyla japonica*, x 10 800; **c**: *Rana nigromaculata*, x 145 000.

The tail contains a 9+2 pattern axoneme without axial rod and undulating membrane (Figs 4d, 5c).

The spermatozoa of the three species have some differences in the neck region. In *R. rugosa*, the pericentriolar material surrounds the centrioles and contains transverse striations (Fig. 3e). However, in *R. dybowskii*, it is only located lateral to the centriole and also has transverse striations. In *R. nigromaculata*, no transverse striations are observed within the pericentriolar material surrounding the centrioles.

DISCUSSION

Comparative ultrastructure of spermatozoa in anurans.

The ultrastructure of spermatozoa is described in six species from four genera of four families (Discoglossidae, Bufonidae, Hylidae and Ranidae) of anurans in this chapter. *B. orientalis*, *B. bufo gargarizans* and *H. japonica* have spermatozoa that conform to the generalized amphibian sperm (see JAMIESON, this volume). On the other hand, *Rana* species have spermatozoa that differ markedly from the above anurans in their organization.

The acrosome is conical in most anurans, but is saccular in *Rana*, and is a coiled structure to one side of the nucleus in *Rhacophorus* [29]. This character of the saccular and coiled acrosome is considered to be apomorphic as compared with the conical acrosome.

The subacrosomal cone present in the sperm of *Ascaphus* [22] is characteristic of urodeles [32, 33] but is not present in any other anurans. JAMIESON *et al.* [22] reported that this structure is a widespread and plesiomorphic feature of amniote sperm.

The endonuclear canal as a main characteristic structure of urodelan sperm has been observed in the primitive anuran families, Ascaphidae [22] and Discoglossidae [18, 23, 39]. This character should be considered as plesiomorphic compared to the other anurans which lack the canal. *Discoglossus* [39] especially has a long canal running the entire length of the nucleus as in the urodeles. In *Bombina* and *Alytes* [18] the canal penetrates roughly to the middle of the nucleus. The perforatorium has been observed in many anurans [12, 14, 18, 22-27, 34, 36-39] and urodeles [8-11, 31-32] but shows differences in its shape and position. The ascaphids [22] and discoglossids [18, 23, 39] have a rod-shaped endonuclear perforatorium which is also characteristic of urodeles. This must be considered plesiomorphic, in comparison with the completely conical extranuclear perforatorium which has been observed in myobatrachids [25], bufonoids [14, 24, 26], hylids [26-27, 37] and leptodactylids [12, 34]. The higher anuran families, Pipidae, Ranidae and Rhacophoridae have no perforatorium [13, 29, 35, 38]. It can thus be deduced that absence of the perforatorium is an apomorphic character in anurans. The perforatorium is progressively simplified and finally disappears in several higher arthropods and vertebrates during evolution of a terrestrial life [6].

The orientation of the centrioles varies depending on the systematic position of the animal [1]. Anurans show three types of orientation of the centrioles. In the first type the distal and proximal centrioles lie at an angle of 70° to each other whereas the proximal centriole and the main axis are perpendicular to each other. This type has been observed in ascaphids [22], discoglossids [18, 23, 39] and myobatrachids [25]. In the second type the two centrioles are perpendicular to each other. This type is seen in bufonoids [14, 24], hylids [27, 37] and leptodactylids [12, 34]. The third type is characterized by the centrioles forming an angle of approximately 140° to each other. This type is seen in pipids [13, 38] and ranids [35].

The tail has been used as an important taxonomic character in many animal groups [1-5, 7, 16, 28]. The tail of most amphibians contains an axoneme, axial rod and undulating membrane. In pipids [13, 38], ranids [35], rhacophorids [29] and the leptodactylid *Telmatobius* [34] the tail has an axoneme only. *Hyla* in the Hylidae [27, 36] has a tail in which the axoneme and axial rod lack an undulating membrane. This tail seems to be the intermediate type in anuran sperm. We thus conclude that a tail consisting of undulating membrane and axial rod is plesiomorphic.

The position of the mitochondria varies in anuran sperm. Mitochondria adjacent to only the axial rod have been observed in ascaphids (termed a paraxonemal rod in this species) [22], discoglossids [18, 23, 39], myobatrachids [25] and urodeles [8-11, 32-33]. In bufonoids [14, 24, 26], hylids [26-27, 37] and leptodactylids [12, 34], they surround the axoneme and constitute a sheath separated by the cytoplasmic canal. The mitochondrial sheath without cytoplasmic canal is observed in pipids [13, 38] and ranids [34]. LEE & JAMIESON [25] suggested in myobatrachids that the location of mitochondria adjacent to only the axial rod is plesiomorphic as compared with the mitochondria surrounding the axoneme.

Phylogenetic relationships in anurans based on spermatozoal ultrastructure.

Table 1 shows phylogenetic trends within the anurans, based on the data of comparative spermatology. The main evolutionary tendencies observed in anurans based on ultrastructural characteristics of spermatozoa are (1) the disappearance of the subacrosomal cone of Ascaphidae in the other anuran groups, (2) a reduction in the length of the endonuclear canal relative to Ascaphidae and Discoglossidae and the eventual disappearance of this structure from the other anuran groups, (3) the disappearance of the perforatorium and the axial rod in Pipidae and

TABLE 1. — Phylogenetic relationships based on the ultrastructure of anuran spermatozoa

PC, plesiomorphic characters; AC, apomorphic characters; Ur, Urodeles; As, Ascaphidae; Di, Discoglossidae; My, Myobatrachidae; Bu, Bufonidae; Hy, Hylidae; Le, Leptodactylidae; Pi, Pipidae; Ra, Ranidae; Rh, Rhacophoridae. *, no data.

		families										
		Ur	As	Di	My	Bu	Hy	Le	Pi	Ra	Rh	
characters			Ascaphus	Discoglossus Alytes Bombina	Limnodynastes Mixophyes Neobatrachus	Bufo	Pachymedusa Litoria Hyla	Odontophrynus Telmatobius	Xenopus	Rana	Rhacophorus	
PC	marginal filament											
	axial rod displaced from axoneme											
	subacrosomal cone											
	ring structure		*									
	endonuclear canal											
	endonuclear perforatorium											
	45°-70° angle of centrioles											
	mitochondrial location related to axial rod											
	undulating membrane											
	axial rod											
	extranuclear perforatorium											
	conical acrosome											
AC	sperm tail with axoneme only											
	140° angle of centrioles										*	
	saccular or lateral acrosome											
	helical spermatozoa											

Ranidae, (4) the disappearance of the undulating membrane in Hylidae, Pipidae and Ranidae, (5) an increase in the angle between the two centrioles: 30°-70° in Ascaphidae, Discoglossidae and Myobatrachidae, 90° in Leptodactylidae, Bufonidae and Hylidae, 140° in Pipidae and Ranidae (Fig. 6).

Anurans studied so far can be divided into three groups with regard to phylogenetic relationships based on the ultrastructure of spermatozoa. The first group includes Ascaphidae and Discoglossidae. They appear drastically isolated from the other anurans because they are characterized by an endonuclear canal, a rod-shaped endonuclear perforatorium, the angle of the centrioles and location of mitochondria adjacent to only the axial rod. The subacrosomal cone appears in only Ascaphidae. These characters show most of the common feature of urodeles. Therefore this group should be considered as plesiomorphic and the Ascaphidae as more plesiomorphic than the Discoglossidae. This interpretation perfectly agrees with the traditional classification. Based on the ultrastructure of acrosome and incompletely condensed nucleus, *Bombina* and *Alytes* are more closely related than they are to *Discoglossus*; *Discoglossus* [39] has the ring structure and the endonuclear canal occupying the whole length of the nucleus which has also been observed in urodeles. With respect to the above characteristics, *Discoglossus* seems to be the most primitive genus among Discoglossidae. However, this interpretation disagrees with the phylogenetic position of three genera from anatomical and karyological data. *Bombina* is the most primitive in an osteological classification [40]. *Alytes* is the most primitive from the karyological point of view in having a karyotype similar to that of primitive urodeles [30].

The second group, including Myobatrachidae, Bufonidae, Hylidae and Leptodactylidae, is characterized by common features: the conical acrosome and the conical extranuclear perforatorium. Myobatrachidae are quite different from this group in having the same structure of the sperm tail as the Ascaphidae and Discoglossidae. These characteristics suggest that Myobatrachidae may occupy the most primitive position in this group. Myobatrachidae has been classified as a subfamily of the family Leptodactylidae. However, recent phylogenies using morphological data [17] or molecular data [20] have recognized myobatrachids as a family separate from the Leptodactylidae. Ultrastructural data of the spermatozoa are in agreement with the latter interpretation [25]. Bufonidae, Hylidae and Leptodactylidae are also united by a single apomorphy, mitochondrial location surrounding the axoneme. This contrasts with the plesiomorphic location of the mitochondria associated with the axial rod in ascaphids [22], discoglossids [18, 23, 39] and urodeles [8-11, 32-33]. The phyletic affinity between these three families is recognized by various authors [19-20, 30].

The third group includes Pipidae, Ranidae and Rhacophoridae. They have common features, such as no perforatorium and a tail with only the axoneme. Although they are different in the morphology of the acrosome, Pipidae and Ranidae are closely related based on the centriolar arrangement as well as the above two characters. The conical acrosome of Pipidae appears as a plesiomorphic character in comparison to the saccular acrosome in Ranidae. This relationship between two families coincides with the morphological data suggested by GRIFFITHS [19]. However, according to MORESCALCHI [30], Pipidae are closely related to the Discoglossidae from karyological data. Rhacophoridae are characterized by the coiled acrosome lying on one side of nucleus.

In anurans the classification based on comparative spermatology is almost similar to traditional classification. Comparative spermatology thus may be considered as a useful new tool for the understanding of anuran phylogeny.

ACKNOWLEDGEMENTS

We are much indebted to Mr. Ku Hwan KIM for printing micrographs and to Jae Hoon HONG for drawing the table. This work has been supported by a grant from Korea Research Foundation to Y. H. LEE.

REFERENCES

1. AFZELIUS, B. A., 1982. — The flagellar apparatus of marine spermatozoa. Evolutionary and functional aspects. In: W. B. AMOS & J. G. DUCKETT, *Prokaryotic & Eukaryotic Flagella*. Cambridge, Cambridge University Press: 495-519.
2. ASA, C. S. & PHILLIPS, D. M., 1987. — Ultrastructure of avian spermatozoa. In: H. MOHRI, *New Horizons in Sperm Cell Research*. Tokyo, Japan Science Societies Press: 365-373.
3. BACCETTI, B., 1970. — The spermatozoon of Arthropoda. IX. The sperm cell as an index of arthropod phylogenesis. In: B. BACCETTI, *Comparative Spermatology*. New York, Academic Press: 169-182.
4. BACCETTI, B. & DALLAI, R., 1976. — The spermatozoon of Arthropoda: XXVII. Uncommon axoneme pattern in different species of Cecidomyid flies. *Journal of Ultrastructure Research*, **55**: 50-69.
5. BACCETTI, B., 1979. — Ultrastructure of sperm and its bearing on arthropod phylogeny. In: A. P. GUPTA, *Arthropod Phylogeny*. New York, Van Nostrand-Reinhold: 609-644.
6. BACCETTI, B., 1979. — The evolution of the acrosomal complex. In: D. W. FAWCETT & J. M. BEDFORD, *The Spermatozoon*. Baltimore & Munich, Urban & Schwarzenberg: 305-329.
7. BACCETTI, B., BURRINI, A. G. & FALCHETTI, E., 1991. — Spermatozoa and relationships in Palaeognath birds. *Biology of the Cell*, **71**: 209-216.
8. BAKER, C. L., 1962. — Spermatozoa of Amphiumae; spermateleosis, helical motility and reversibility. *Journal of the Tennessee Academy of Science*, **37**: 23-39.
9. BAKER, C. L., 1963. — Spermatozoa and spermateleosis in *Cryptobranchus* and *Necturus*. *Journal of the Tennessee Academy of Science*, **38**: 1-11.
10. BAKER, K. R. & BIESELE, J. J., 1967. — Spermateleosis of a salamander *Amphiuma tridactylum* Cuvier. *La Cellule*, **67**: 91-118.
11. BAKER, K. R. & BAKER, C. L., 1969. — Urodele spermateleosis; a comparative electron microscope study. In: B. BACCETTI, *Comparative Spermatology*. New York, Academic Press: 81-87.
12. BAO, S. N., DALTON, G. C. & DE OLIVEIRA, S. F., 1991. — Spermiogenesis in *Odontophrynus cultripes* (Amphibia, Anura, Leptodactylidae); Ultrastructural and cytochemical studies of proteins using E-PTA. *Journal of Morphology*, **207**: 303-314.
13. BERNARDINI, G., STIPANI, R. & MELONE, G., 1986. — The ultrastructure of *Xenopus* spermatozoon. *Journal of Ultrastructure and Molecular Structure Research*, **94**: 188-194.
14. BURGOS, M. H. & FAWCETT, D. W., 1956. — An electron microscope study of spermatid differentiation in the toad, *Bufo arenarum* Hensel. *Journal of Biophysical and Biochemical Cytology*, **2**: 223-240.
15. DALLAI, R. & MAZZINI, M., 1983. — Spermatozoa and Diptera phylogeny. In: J. ANDRÉ, *The Sperm Cell*. The Hague, Martinus Nijhoff: 440-445.
16. DALLAI, R., BELLON, P. L., LANZAVECHIA, S. & AFZELIUS, B. A., 1993. — The dipteran sperm tail; ultrastructural characteristics and phylogenetic considerations. *Zoologica Scripta*, **22**: 193-202.
17. DUELLMAN, W. E. & TRUEB, L., 1986. — *Biology of Amphibians*. New York, McGraw-Hill.
18. FURIERI, P., 1975. — The peculiar morphology of the spermatozoon of *Bombina variegata* (L.). *Monitore Zoologico Italiano*, **9**: 185-201.
19. GRIFFITHS, I., 1963. — The phylogeny of the Salientia. *Biological Review*, **38**: 241-292.
20. HILLIS, D. M., 1991. — The phylogeny of amphibians; current knowledge and the role of cytogenetics. In: D. M. GREEN & S. SESSION, *Amphibian Cytogenetics and Evolution*. New York, Academic Press.
21. JAMIESON, B. G. M., 1987. — *The Ultrastructure and Phylogeny of Insect Spermatozoa*. Cambridge, Cambridge University Press: 1-309.
22. JAMIESON, B. G. M., LEE, M. S. Y. & LONG, K., 1993. — Ultrastructure of the spermatozoon of the internally fertilizing frog *Ascaphus truei* (Ascaphidae: Anura: Amphibia) with phylogenetic considerations. *Herpetologica*, **49**: 52-65.
23. KWON, A. S. & LEE, Y. H., 1992. — The fine structure of spermatozoa in *Bombina orientalis* (Anura, Amphibia). *Nature and Life*, **22**: 15-22.
24. KWON, A. S., KIM, H. J. & LEE, Y. H., 1993. — Fine structure of the neck of spermatozoa and spermiogenesis in *Bufo bufo gargarizans* (Amphibia, Anura). *Nature and Life*, **23**: 95-105.
25. LEE, M. S. Y. & JAMIESON, B. G. M., 1992. — The ultrastructure of the spermatozoa of three species of myobatrachid frogs (Anura, Amphibia) with phylogenetic considerations. *Acta Zoologica (Stockholm)*, **73**: 213-222.
26. LEE, M. S. Y. & JAMIESON, B. G. M., 1993. — The ultrastructure of the spermatozoa of bufonoid and hyloid frogs (Anura, Amphibia): implication for phylogeny and fertilization biology. *Zoologica Scripta*, **22**: 309-323.

27. LEE, Y. H. & KWON, A. S., 1992. — Ultrastructure of spermiogenesis in *Hyla japonica* (Anura, Amphibia). *Acta Zoologica (Stockholm)*, **73**: 49-55.
28. MATTEI, X., 1988. — The flagellar apparatus of spermatozoa in fish. Ultrastructure and evolution. *Biology of the Cell*, **63**: 151-158.
29. MIZUHIRA, V., FUTAESAKU, Y., ONO, M., UENO, J., YOKOFUJITA, J. & OKA, T., 1986. — The fine structure of the spermatozoa of two species of *Rhacophorus* (*arboreus*, *schlegelii*). I Phase-contrast microscope, scanning electron microscope, and cytochemical observations of the head piece. *Journal of Ultrastructure Research*, **96**: 41-53.
30. MORESCALCHI, A., 1973. — Amphibia. In: A. B. CHIARELLI & E. CAPANNA, *Cytotaxonomy and Vertebrate Evolution*. New York, Academic Press: 233-347.
31. NICANDER, L., 1970. — Comparative studies on the fine structure of vertebrate spermatozoa. In: B. BACCETTI, *Comparative Spermatology*. New York, Academic Press: 47-56.
32. PICHÉRAL, B., 1967. — Structure et organisation du spermatozoïde de *Pleurodeles walilii* Michah (Amphibien, Urodèle). *Archives de Biologie*, **78**: 193-221.
33. PICHÉRAL, B., 1979. — Structural, comparative and functional aspects of spermatozoa in urodeles. In: D. W. FAWCETT & J. M. BEDFORD, *The Spermatozoon*. Baltimore & Munich, Urban & Schwarzenberg: 267-287.
34. PISANO, A. & ADLER, R., 1968. — Submicroscopical aspects of *Telmatobius hauthali schreiteri* spermatids. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, **87**: 345-349.
35. POIRIER, G. R. & SPINK, G. C., 1971. — The ultrastructure of testicular spermatozoa in two species of *Rana*. *Journal of Ultrastructure Research*, **36**: 455-465.
36. PUGIN-RIOS, E., 1980. — *Étude comparative sur la structure du spermatozoïde des Amphibiens Anoures. Comportement des gamètes lors de la fécondation*. Thèse, Université de Rennes, Rennes, France.
37. RASTOGI, R. K., BAGNARA, J. T., IELA, L. & KRASOVICH, M. A., 1988. — Reproduction in the Mexican leaf frog, *Pachymedusa dacnicolor*. IV. Spermatogenesis ; a light and ultrasonic study. *Journal of Morphology*, **197**: 277-302.
38. REED, S. C. & STANLEY, H. P., 1972. — Fine structure of spermatogenesis in the South African clawed toad *Xenopus laevis* Daudin. *Journal of Ultrastructure Research*, **41**: 277-295.
39. SANDOZ, D., 1974. — Development of the neck region and the ring during spermiogenesis of *Discoglossus pictus* (Anura Amphibia). In B. A. AFZELIUS, *The Functional Anatomy of the Spermatozoon*. Oxford, Pergamon Press: 237-247.
40. SLABBERT, G. K. & MAREE, W. A., 1945. *Annale Universiteit Stellenbosch*, **23**: 91-97.